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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 10/699,511
Filing Date: October 31, 2003
Appellant(s): BENNETT ET AL.

Tamsen Valoir
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed April 11, 2008, appealing from the Office action mailed July 26, 2007.

(1) Real Party in Interest

A statement identifying by name the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(3) Status of Claims

The statement of the status of claims contained in the brief is correct.

(4) Status of Amendments After Final

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

(5) Summary of Claimed Subject Matter

The summary of claimed subject matter contained in the brief is correct.

(6) Grounds of Rejection to be Reviewed on Appeal

The appellant's statement of the grounds of rejection to be reviewed on appeal is correct.

(7) Claims Appendix

The copy of the appealed claims contained in the Appendix to the brief is correct.

(8) Evidence Relied Upon

5,851,808 Elledge et al. 12-1998

Watson et al. "Cloning and Assembly of PCR Products Using Modified Primers and DNA Repair Enzymes" BioTechniques, vol. 23 no. 5 (1997), pp. 858-862.

Stahl et al. "Solid-Phase Gene Assembly of Constructs Derived from the Plasmodium falciparum Malaria Blood-Stage Antigen Ag332" BioTechniques, vol. 14 no. 3 (1993), pp. 424-436.

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-7 are rejected under 35 U.S.C. 103(a) as being unpatentable over Watson et al. (BioTechniques, 1997) and Elledge et al. (USPN 5,851,808) in view of Stahl et al. (BioTechniques, 1993).

With regard to claim 1, Watson et al. teach a method of assembling PCR fragments comprising (see p. 858 the abstract and p. 860 col. 3 under cloning of lac operon fragment):

a) making a first PCR fragment with first and second primers, wherein the second primer comprises a modified nucleotide that can be removed by a DNA repair enzyme, resulting in a 3' overhang (see p. 858 the abstract and p. 860 col. 3 under cloning of lac operon fragment);

b) treating the first PCR fragment with a DNA repair enzyme to generate a 3' overhang

c) making a second PCR fragment with third and fourth primers, wherein the third and fourth primers each comprises a modified nucleotide that can be removed by a DNA repair enzyme resulting in a 3' overhang (see p. 858 the abstract and p. 860 col. 3 under cloning of lac operon fragment);

d) treating the second PCR fragment with a DNA repair enzyme to generate a 3' overhang (see p. 858 the abstract and p. 860 col. 3 under cloning of lac operon fragment);

e) annealing and ligating the first and second PCR fragments (see p. 858 the abstract and p. 860 col. 3 under cloning of lac operon fragment);

f) optionally repeating steps c, d and e until a last PCR fragment is added to the

growing chain to produce an assembled fragment (see p. 858 the abstract and p. 860 col. 3 under cloning of lac operon fragment),

g) circularizing the assembled fragment (see p. 860 col. 3 under cloning of lac operon fragment, where the fragment is circularized in the vector before transformation)

With regard to claim 2, Watson et al. teach one of the PCR fragments comprises an origin of replication and a selectable marker (see p. 860 col. 3 under cloning of lac operon fragment, the lac operon contains a selectable marker and the vector contains an origin of replication).

With regard to claim 3, Watson et al. teach the first PCR fragment or the last PCR fragment comprises an origin of replication and a selectable marker (see p. 860 col. 3 under cloning of lac operon fragment, the lac operon contains a selectable marker and the vector contains an origin of replication).

With regard to claim 5, Watson et al. teach the nucleotide is deoxyuridine and the DNA repair enzyme is Uracil-DNA-glycosylase followed by T4 endonuclease V (see p. 858 first full paragraph under introduction).

With regard to claims 6 and 7 Watson et al. teach the assembled DNA is greater than 30 kb see p. 860 col. 3 under cloning of lac operon fragment where the lac operon and the vector are greater than 30 kb).

With regard to step (a) of claim 1, Watson et al. do not teach using site specific recombination.

With regard to step (g) of claim 1, Watson et al. do not teach circularization with a site specific recombinase.

With regard to steps (a) and (g) of claim 1, Elledge et al. teach site specific recombination and circularization occurring simultaneously in a single step, with recombinase (see col. 17 lines 44-64, where Elledge teach site specific recombination with cre recombinase *in vitro*. By

employing the Cre/lox system for recombination of two plasmids, Elledge necessarily teaches simultaneous circularization and recombination of the plasmid).

One of ordinary skill in the art at the time the invention was made would have been motivated to apply the method of using the cre/lox recombinase system as taught by Elledge with the method of DNA assembly as taught by Watson in order to reduce the time and effort associated with restriction mediated DNA assembly. Elledge et al. teach site specific recombination eliminates the use of restriction enzymes and DNA ligase: instead, these functions are both carried out simultaneously by a single enzyme Cre. Additionally, Elledge teach site specific recombination using Cre in an *in vitro* system. It would have been prima facie obvious to apply the cre/lox recombinase system as taught by Elledge with the method of DNA assembly as taught by Watson in order to have increased efficiency in assembling DNA fragments. The use of cre/lox recombinase system provides for rapid and efficient generation and manipulation of recombinant DNA.

With respect to step (b) of claim 1, Watson et al. and Elledge et al. do not teach immobilizing the PCR fragments for assembly.

With regard to step (g) of claim 1, Watson et al. and Elledge et al. do not teach removing the assembled fragment from the solid support.

Stahl et al. teach immobilizing PCR fragments for assembly (see p. 424 abstract and p. 425 Figure 1).

Stahl et al. teach subsequently removing the assembled gene construct from the bead prior to subcloning (see p. 426 col. 2 first full paragraph).

One of ordinary skill in the art at the time the invention was made would have been motivated to apply the step of immobilizing the fragments for assembly as taught by Stahl with the method of DNA assembly as taught by Watson and Elledge in order to have a controlled

assembly of the fragments. Stahl et al. state, "Immobilization of the first oligonucleotide enables controlled stepwise annealing/ligation of successive 5' phosphorylated oligonucleotides to rapidly build up accurate gene constructs making it possible to sub clone for subsequent expression of the gene product (see p. 424 col. 3 first full paragraph)." It would have been prima facie obvious to apply the step of immobilizing the fragments for assembly as taught by Stahl with the method of DNA assembly as taught by Watson and Elledge in order to stabilize and control the assembly of the gene constructs. Controlled assembly yields more accurate gene constructs.

(10) Response to Argument

Cited Art

Appellants describe the prior art cited and the deficiencies of each piece of prior art. Appellants are reminded the rejection of the claims is made under 35 USC 103 (a) and not 35 USC 102, therefore each piece of art need not teach each limitation of each claim but rather the total of the teachings of each piece of the prior art would lead a skilled artisan to reason that the practice of the instant claims is obvious.

Nothing is Missing in the Cited Art

Appellants argue, nowhere in the cited art is a recombinase used on a solid support, only that simple enzymes such as restriction endonucleases and ligases are disclosed as used with a solid support. Neither of which are topologically sensitive enzymes. Appellants assert that restriction endonucleases and ligases are simple enzymes and that Cre recombinase is a topologically sensitive enzyme. This assertion is not persuasive because both restriction endonucleases and ligases are topologically sensitive to the same extent as Cre recombinase. Restriction endonucleases and ligases require nucleotide specificity to function and also require these specific sites be accessible to the enzyme so that it may function. This is also the requirement of Cre recombinase. The loxP sites are specific sites required for activity and these sites must be accessible to the enzyme so that it may function. Therefore, restriction

endonucleases and ligases are analogous to the Cre recombinase with respect to topological sensitivity requirements.

Appellants argue there is no suggestion in the art of a reasonable expectation of success of using a recombinase on a solid support. This argument is not persuasive because the rejection above outlines the motivation for combining the teachings of Watson, Elledge and Stahl. Elledge et al. teach the Cre/lox recombinase system, Watson et al. teach assembly of PCR fragments and Stahl et al. teach gene assembly on a solid support. Elledge et al. teach the advantages of using the Cre/lox recombinase system are that site specific recombination eliminates the use of restriction enzymes and DNA ligase: instead, these functions are both carried out simultaneously by a single enzyme Cre. Stahl et al. teach the advantages of assembly on a solid support are that immobilization of the first oligonucleotide enables controlled stepwise annealing/ligation of successive 5' phosphorylated oligonucleotides to rapidly build up accurate gene constructs making it possible to sub clone for subsequent expression of the gene product. In view of the advantages of the Cre/lox recombinase system disclosed by Elledge et al. and the advantages of assembly on a solid support disclosed by Stahl et al. a skilled artisan would be motivated to use the Cre/lox recombinase system of Elledge et al. with the solid support of Stahl et al. when assembling PCR fragments as disclosed by Watson et al.

Additionally, there is a reasonable expectation of success as the legal standard for "reasonable expectation of success" is provided by caselaw and is summarized in MPEP 2144.08, which notes "obviousness does not require absolute predictability, only a reasonable expectation of success; i.e., a reasonable expectation of obtaining similar properties. See , e.g. , In re O'Farrell , 853 F.2d 894, 903, 7 USPQ2d 1673, 1681 (Fed. Cir. 1988)." In this factual case, there is express suggestion in the prior art that PCR products can be assembled by Watson. There is further evidence as shown by Stahl that assembly can occur on a solid support. Finally Elledge teaches that Cre recombinase provides simultaneous recombination and circularization of plasmid

DNA. This sufficient for a reasonable expectation of success. The MPEP cites In re O'Farrell, which notes regarding "obvious to try" at page 1682, that,

> "In some cases, what would have been "obvious to try" would have been to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful. E.g ., In re Geiger , 815 F.2d at 688, 2 USPQ2d at 1278; Novo Industri A/S v. Travenol Laboratories, Inc ., 677 F.2d 1202, 1208, 215 USPQ 412, 417 (7th Cir. 1982); In re Yates , 663 F.2d 1054, 1057, 211 USPQ 1149, 1151 (CCPA 1981); In re Antonie , 559 F.2d at 621, 195 USPQ at 8-9. In others, what was "obvious to try" was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it. In re Dow Chemical Co ., 837 F.2d, 469, 473, 5 USPQ2d 1529, 1532 (Fed. Cir. 1985); Hybritech, Inc. v. Monoclonal Antibodies, Inc ., 802 F.2d 1367, 1380, 231 USPQ 81, 90-91 (Fed. Cir. 1 986), cert. denied , 107 S.Ct. 1606 (1987); In re Tomlinson ; 363 F.2d 928, 931, 150 USPQ 623, 626 (CCPA 1966).

The court in O'Farrell then, affirming the rejection, notes "Neither of these situations applies here." For the instant case, it is clear that neither situation applies here either. This is not a situation where the prior art suggests varying a variety of parameters, since the prior art directly points to the assembly of PCR products by Watson, that assembly can occur on a solid support by Stahl and that Cre recombinase provides simultaneous recombination and circularization of plasmid DNA by Elledge. This is also not a situation where only general guidance was given. The prior art provides specific guidance directing the assembly of DNA fragments on a solid support, recombining and circularizing the DNA as discussed in the rejection and as taught by Watson, Elledge and Stahl.

Prima Facie Obviousness Case Has Been Shown and the Proper Limitations are Addressed.

Appellants argue a prima facie case has not been made. Specifically, Appellants argue none of the cited art meets the limitation of "simultaneously removing and circularizing the assembled fragment from the solid support with a site specific recombinase in a single step" Appellants argue there is no mention of using recombinases on solid supports in the cited art and that the Office's argument is recombinases are in the art and solid supports are in the art therefore using recombinases on solid supports is in the art.

This is a mischaracterization of the Office's position. As outlined in the rejection Stahl et al. successfully employs restriction endonucleases and ligases with assembled gene products immobilized on a solid support. Stahl does not use a site specific recombinase. Elledge et al. uses the site specific recombinase system of Cre/lox in in vitro applications and discloses the advantages of such a system. It is the position of the Office a skilled artisan would recognize the advantages of the Cre/lox system and being aware of the success of Stahl et al. in using restriction endonucleases and ligases with assembled gene products immobilized on a bead would then be motivated to use the Cre/lox system with DNA constructs immobilized on a solid support. The artisan would have a reasonable expectation of success because both restriction endonucleases and ligases are analogous to Cre recombinase. Restriction endonucleases and ligases require nucleotide specificity to function and also require these specific sites be accessible to the enzyme so that it may function. This is also the requirement of Cre recombinase. The loxP sites are specific sites required for activity and these sites must be accessible to the enzyme so that it may function. Therefore the skilled artisan would have a reasonable expectation that the Cre/lox system would behave as restriction endonucleases and ligases and that the Cre/lox system would work with a solid support as did the restriction endonucleases and ligases disclosed by Stahl et al.

There is a Reasonable Expectation of Success and Mere Arguments are not used as Evidence

Appellants argue the Office failed to provide reasonable expectation of success. This argument is not persuasive because the teachings of Stahl et al. provide the basis for reasonable expectation of success. Stahl et al. teach that restriction enzymes and ligases can be successfully used with DNA constructs immobilized to a solid support. Both restriction enzymes and ligases are topologically sensitive in that both of these enzyme have site (sequence) specific requirements and the specific sequence must be accessible to the enzymes for them to function properly. If the recognition sequence for a specific restriction endonuclease was not readily accessible to the endonuclease the enzyme would not function properly. Stahl et al. were aware of this and ensured that when the DNA was immobilized to the support that the specific sites required by the enzyme to function were available and accessible to the enzyme. The successful use of restriction enzymes and ligases by Stahl evidence that immobilizing DNA to a solid support does not markedly alter the structure such that enzymes which require specific sequences and accessibility to those sequences can no longer function as expected. A skilled artisan would recognize analogous parameters would hold true for the Cre recombinase and if the parameters were properly controlled for would have a reasonable expectation of success as did Stahl et al.

Recombinases are Topologically Sensitive

Appellants argue recombinases are topologically sensitive and assert this fact has not been rebutted. This fact is not in contention. However, it is argued that restriction endonucleases and ligases are also topologically sensitive. Both restriction endonucleases and ligases are topologically sensitive to the same extent as Cre recombinase. Restriction endonucleases and ligases require nucleotide specificity to function (i.e. are site specific) and also require these specific sites be accessible to the enzyme so that it may function. This is also the requirement of Cre recombinase. The loxP sites are specific sites required for activity and these sites must be

accessible to the enzyme so that it may function. Therefore, restriction endonucleases and ligases are analogous to the Cre recombinase with respect to topological sensitivity requirements. Additionally, as discussed above the successful use of restriction enzymes and ligases by Stahl evidence that immobilizing DNA to a solid support does not markedly alter the structure/architecture of the DNA such that enzymes which require specific sequences and accessibility to those sequences can no longer function as expected.

Declaratory Evidence does not Show that Topologically Sensitive Recombinases are not Expected to Function on Substrates Tethered to a Solid Support.

Appellants argue the declaration shows recombinases are not expected to function on tethered to solid supports. In fact the declaration offers no evidence of this except for the opinion of Dr. Bennet, a named inventor. Dr. Bennet states that immobilized DNA has a different topological structure than either native DNA in vivo or purified DNA in vitro and therefore because the Cre/lox reaction changes the topological structure of the DNA substrate that a DNA structure tethered to a solid support might not undergo the conformation changes required for recombination. Appellants submitted several papers in support of this assertion. However, none of these papers address or support the assertion that by binding DNA to a solid substrate the structure is changed so markedly that recombinase would not function. Appellants argue Dr. Bennet, a person of ordinary skill in the art declared under penalty of law that he would not predict that recombinases would function immobilized on a solid support. The office does not rebut the fact that Dr. Bennet would not predict that recombinases would function immobilized on a solid support. However, again there is no evidence to support the assertion by Dr. Bennet that by binding DNA to a solid substrate the structure is changed so markedly that recombinase would not function. This prediction is merely the opinion of Dr. Bennet, not a fact.

As discussed previously restriction enzymes and ligases are also topologically sensitive to some extent. These enzymes require the structure of DNA to be such that the specific sites are

available and accessbile for the enzyme. For these enzymes to bind the DNA and perform their respective functions the DNA must also be free to undergo certain comformation changes. Immobilizing DNA to a solid support did not alter the structure or archetecture of the DNA such that these enzymes would no longer function. Therefore in the absence of evidence to support Dr. Bennet's assertion that binding DNA to a solid substrate the structure is changed so markedly that recombinase would not function, the declaration was not found to be persuasive. The declaration was not supported by any evidence showing a marked alteration in the structure of DNA once immobilized to a solid support. Therefore the conclusion that recombinase would not function on DNA immobilized to a solid support could not be made and it is the Office's position that no legal error was made in the evaluation of the declaration.

No Evidence has been unchallanged

All of Appellants' arguments have been sufficiently addressed and a proper prima facie case for obviousness has been made.

(11) Related Proceeding(s) Appendix

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

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